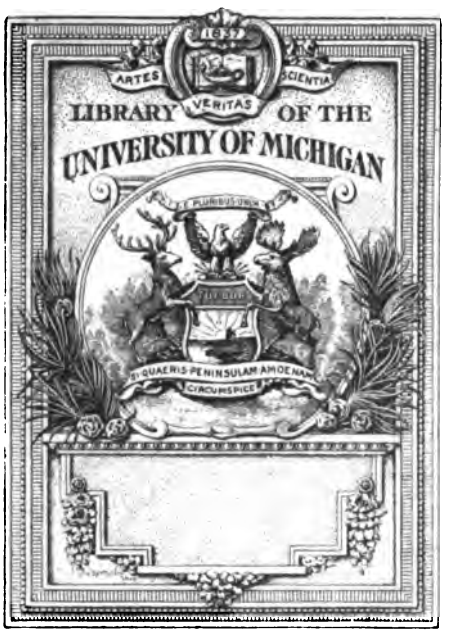
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THE EMBRYOLOGY OF CREPIDULA

BY

EDWIN GRANT CONKLIN

An Abstract of a Dissertation presented to the Board of University Studies of the Johns Hopkins University for the Degree of Doctor of Philosophy, June 11, 1891, and afterwards incorporated in a Memoir on "The Embryology of Crepidula, a Contribution to the Cell Lineage and Early Development of Some Marine Gasteropods."

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THE

EMBRYOLOGY OF CREPIDULA

A CONTRIBUTION TO THE CELL LINEAGE AND EARLY DEVELOPMENT OF SOME MARINE GASTEROPODS

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A. INTRODUCTION.

1. *Purpose and History of the Work.*

THE purpose of the following work from its inception has been to make as careful a study as possible of the cleavage of the ovum, the formation of the germinal layers and definitive organs, and the axial relations of the ovum to the larval and adult axes. At the time when this work was begun, several years ago, scarcely any attempts had been made to trace the history of individual blastomeres through the entire development to the formation of definite organs. The early stages of cleavage had received a great deal of attention, but the later stages had been largely neglected; and although the origin and homology of the germ layers was perhaps the most frequently discussed subject in embryology, yet the relation of these layers to the individual blastomeres of the cleaving ovum had been determined in comparatively few cases. Since that

time a number of very valuable papers have appeared on this subject of "cell lineage," as Wilson ('92) has aptly termed it. The results of such work are no longer as novel as they were four or five years ago, and yet the general interest in the subject has greatly increased, and that, too, in spite of the fact that there is a growing school of biologists who believe that individual blastomeres have no necessary relation to future organs. The subject of germ layers is no longer so important as it was once considered; in fact, the theory of the homology of the germinal layers has met with so many difficulties of late that it is now generally maintained only in a greatly modified form. However, the fundamental idea which was prominent in germ-layer discussions is of vital interest to-day. In the whole history of the germ-layer theories I see an attempt to trace homologies back to their earliest beginnings. This problem is as important to-day as it ever was, and whether one find these earliest homologies in layers or regions or blastomeres or the unsegmented ovum itself, the quest is essentially the same.

Within this question of the earliest homologies is included another of great present interest, *viz.*, the significance of cleavage. Is it an orderly sifting of materials, a "mosaic work," or, as Driesch ('93) has maintained in the case of the echinids, a mere quantitative division of homogeneous material? Can the cells of cleaving eggs be compared with each other as the organs of adult animals can? Can one properly speak of the homology of blastomeres? Are the chief axes and regions of the egg or embryo homologous in different animals? And finally, are the causes of the various forms of cleavage to be found primarily in the constitution of the egg itself, in other words, in the internal conditions, or rather in the external conditions, such as pressure, surface tension, gravity, etc.? I know that in these days, when "all the world shakes eggs," it may be hazardous to risk an opinion on these questions which is not based on experimental work. And yet, while fully recognizing the value of experimental embryology, we ought not to forget that "Nature is continually performing some very remarkable experiments in her own way," and I believe we need to know

more about these normal processes before we can properly understand abnormal ones. In order to know the significance of cleavage, it is necessary not only to find out how much the egg may be fragmented or the blastomeres transposed without irreparably destroying development, but also and much more, it is necessary to know every step in the normal formation of the embryo. It is less important to know what remedial processes Nature may have for healing broken eggs, than to understand her usual methods of developing unbroken ones. Whether and how much this "secondary," or regenerative development may differ from the "primary," or normal, is still an open question. If there be a difference, as Roux ('93) maintains, the phenomena of regenerative or secondary development are much more complicated and difficult of explanation than the process of primary or normal development, since in these cases we have to explain the phenomena of normal development plus those of regeneration. In any case the phenomena of normal development are the ones to be explained, whatever method may be used ; and before any explanation can be given it is necessary to know the usual development as thoroughly as possible.

It is because of the perennial interest in these questions of the earliest homologies, and of the significance and causes of the various forms of cleavage, and also with the hope that I may be able either directly or indirectly to add something, however little, to the solution of some of these problems, that I now bring forward this long-delayed contribution on the Embryology of Crepidula.

Crepidula is a genus of prosobranchiate gasteropods, whose development has never heretofore been studied so far as I can learn, — a genus, moreover, which is in many respects a very interesting one, apart from its embryology ; besides, it is so abundant all along our Atlantic coast from Labrador to Florida, and its eggs are so easily obtained, so numerous, and so exceedingly favorable for embryological research, that it seems remarkable that no one has hitherto attempted to study its development.

This work was begun in the summer of 1890, while I was occupying the Johns Hopkins University table at the Marine Laboratory of the United States Fish Commission at Wood's Holl, Mass. During the succeeding winter I continued the work in Professor Brooks' laboratory at Baltimore, and in the summer of 1891 I again occupied the Johns Hopkins table at Wood's Holl, and continued to work on the same subject. Since that time my work has suffered long and repeated interruptions owing to the pressure of other duties.

I had hoped to be able to present in one paper both the earlier and the later stages in the development, but the work has grown so much, both in extent and difficulties, that it has seemed best to publish the results of investigations on the early stages first, and to supplement these by another paper on the later stages as soon as possible. Since the study of the later stages is less general in its bearing and more specifically applicable to the Mollusca, such a division of the subject will not be an illogical nor an unwelcome one. Two preliminary papers have been published on this subject, — one on the general embryology of Crepidula and Urosalpinx (Conklin, '91), the other on the cleavage in Crepidula ('92).

During the first year of the work my attention was directed exclusively to the development of Crepidula fornicata, and a large number of drawings of the various stages in the embryology of this species were made; for this reason it forms the chief subject of this paper, although in some respects C. plana is a more favorable object for study. It was not until the summer of 1892 when, through the courtesy of Professor Whitman, I was enjoying the privileges of the Marine Biological Laboratory at Wood's Holl, that I obtained material for the study of the embryology of C. plana and C. convexa. I have, however, made a careful comparison of the development of these three species, and in most respects have found the cleavage and formation of the germ layers and larval organs very similar in all of them.

Through the kindness of my friend and former pupil, Mr. Harold Heath of the Leland Stanford University, I have recently received a number of adult specimens and a good col-

lection of eggs and embryos of C. adunca, a species quite common on the Pacific coast. I have made a brief study of the embryology of this form. The peculiar features in its development will be referred to later. During the course of this work I have also studied, more or less carefully, the embryology of several other genera of marine prosobranchs, *viz.*, Urosalpinx cinerea, Fulgur carica, Sycotypus canaliculatus, Illyonassa obsoleta, Tritia trivittata, Neverita duplicata.

If space and opportunity permitted, it would be a pleasure to mention the names of many friends who in one way or another have assisted me, but I cannot fail to speak of two or three persons who have placed me under very great obligations. I am indebted to Professor C. O. Whitman, Director of the Marine Biological Laboratory, for the opportunity of working at that excellent institution, as well as for many stimulating suggestions and friendly criticisms ; to Professor W. K. Brooks, my former instructor, for valuable assistance during the first year of my work ; and particularly am I indebted to my wife, who has finished from my camera sketches many of the drawings which illustrate this paper, and has in many other ways rendered me great assistance.

2. *Methods.*[1]

The ova were fixed in many different fluids, — Kleinenberg's picro-sulphuric, picric acid in sea water, Perenyi's, Flemming's stronger and weaker, Merkel's, Auerbach's, Hermann's, corrosive sublimate, chromo-formic, chromo-acetic and absolute alcohol ; but for surface views of the entire egg none of these methods for a moment compares with the first named, *i.e.*, Kleinenberg's stronger picro-sulphuric. The ova were left in this for a length of time varying from fifteen to thirty minutes, and were then gradually transferred to 70 % alcohol. They were left in this until all traces of picric acid had been washed out, and were finally preserved in 95 % alcohol.

[1] The substance of this section was published in the American Naturalist, vol. XXVII (1893).

As a result of many experiments with almost every one of the common staining fluids, I found that the best method of preparing surface views of the whole egg or embryo was the following: (1) Transfer the object gradually from alcohol to water. (2) Stain from five to ten minutes in a solution of Delafield's (Grenacher's) haematoxylin diluted about six times with distilled water and rendered *slightly* acid by a trace of HCl. (3) De-hydrate and clear in oil of cedar or xylol. (4) Mount in balsam, supporting the cover glass so as to prevent crushing. By occasionally softening the balsam with a drop or two of xylol and slightly moving the cover glass the objects can be rolled into any position desired.

By this method wonderfully beautiful surface preparations were obtained, showing with remarkable clearness not only the nuclei and cell boundaries, but also the karyokinetic figures, and in many cases the archoplasmic spheres and centrosomes. One very considerable advantage of this method is that the preparations are permanent — in fact during the first year or two they become better with age instead of degenerating. Most of the preparations from which the figures were drawn are still in existence, and can be consulted at any time.

I have employed this method with almost as good results in the preparation of surface views of the embryo chick and English sparrow, and also with considerable success on other molluscan eggs and embryos, as well as those of annelids and echinoderms.

The objects for sectioning were fixed in various fluids, some of which showed certain points of structure better than others ; for general purposes, however, excellent results were obtained by fixing in the picro-sulphuric solution, though the chromatic filaments and individual chromosomes were brought out much more clearly by the use of absolute alcohol, and the spindle fibres and centrosomes were more clearly shown by the use of Flemming's or Hermann's fluid. In all cases the objects were imbedded in paraffin, and the best results were obtained by staining on the slide. On the whole I have found a double stain, consisting of Delafield's haematoxylin followed by a solution of erythrosine in aniline water, to give the best re

sults, though many other stains were useful, particularly the Biondi-Erlich mixture and the iron haematoxylin of Heidenhain.

One other thing ought to be mentioned in this connection. I have in no instance been able to follow any one lot of eggs throughout any considerable part of their development. When removed from the mantle cavity of the mother they do not develop normally for more than two or three days. I tried keeping some of the eggs in small dishes, changing the water twice a day; others were placed in a large jar, in which the water was continually aerated by a stream of air; still others were placed in a jar, the mouth of which was covered by silk netting, and the jar was then inverted in a tank of flowing water; the most successful method, however, was to put the eggs in open bottles, which were then placed in an aquarium through which water was constantly flowing. Yet by none of these methods could the eggs be kept normal for more than a few days. It would seem that the circulation of water within the mantle chamber of the mother is more perfect and gentle than could be obtained by any method which I could devise. It was necessary, therefore, to take eggs from a large number of individuals in order to get a complete series, since all the eggs laid by one individual are in nearly the same stage of development. Fortunately, there are such vast numbers of fertile females during the breeding season as to make this an easy task.

B. THE GENUS CREPIDULA.

1. *Natural History.*

At least three species of the genus Crepidula are found on the Atlantic coast of the United States,[1] *viz.*, C. fornicata Lam., C. plana Say, and C. convexa Say, all of which are quite abundant along the shores of New England. All these species are more or less completely sedentary, and they show the most remarkable individual differences in the shape of their shells due

[1] Other species have been described, *viz.*, C. unguiformis Stimson, C. glauca Say, C. acuta Lea. Concerning the first of these there is no doubt that it is identical with C. plana, and I am convinced after a careful anatomical and embryological examination of the last two that they are only local varieties of C. convexa (cf. Verrill '74) Invertebrate Animals of Vineyard Sound.

to the character of the surfaces upon which they are attached. Upon a smooth, plane surface the shell is regular and unusually broad and flat; on a convex surface it is deep and highly arched; on a concave surface it is concave, sometimes to the extent of being almost semicircular; on a twisted surface, like the columella of Neverita, it is twisted; on an irregular surface, such as a rough stone, it is irregular; if pressed upon from the sides the animal and shell become long and narrow; if growth is limited in front the shell becomes short and broad; if limited on all sides the shell may increase greatly in thickness but remains small, filling the space in which it is found. In such cases the lines of growth are crowded closely together and the very edge of the shell may be as thick as any other portion. In small places, such as the interior of Illyonassa shells, C. plana may be dwarfed to one twenty-fifth the size of normal specimens. These individual variations in the shape and size of the animals and shells appear in all the species of Crepidula, but they are most marked in C. plana. The cause of the variations in the *shape* of the shells is not far to seek, though the great differences in the *size* of individuals is more difficult to understand: the shape of the shell is conditioned by the shape and position of the mantle edge; the mantle is moulded over the surface upon which the animal rests; and consequently the shape of the shell comes in time to correspond to any sort of a surface upon which the animal is attached.

C. fornicata, the "slipper limpet" or "boat shell," is a common object to all visitors at the sea-shore. It occurs in great numbers on the shells of the king crab, Limulus polyphemus, where it is firmly attached to the ventral side of the carapace and abdomen; sometimes it is found on the appendages, the gill plates, or even the dorsal surface of the crab. After it has reached a certain size, about half that of the adult, it never moves about. It thenceforth leads a perfectly passive existence, being carried about by the king crab, and obtaining all its food by merely sweeping into its mantle chamber currents of water containing particles of food, which are in large part the crumbs which have fallen from the king crab's table. This species is also found abundantly on muddy sea bottoms a short distance

below low-water mark, where it usually occurs in curious chains often containing ten or twelve individuals. In these chains the foot of one individual is firmly fastened to the dorsal side of the shell of the next one, and the heads of all the animals are turned in the same general direction; the first or oldest individual in a chain is usually attached by its foot to a stone or dead shell. Even those which live upon Limulus sometimes show a tendency to pile one upon another, though in this case there are seldom more than two or three in a pile. C. fornicata also occurs, but in comparatively small numbers, on submerged portions of stones, buoys, and wharves. In none of these cases, however, is it able to change its position after it is about half grown, and it obtains all its food from the particles which float to it in the water. The fact that the large Crepidulas are immovably fixed to one spot is shown not only by the shells, which have in many cases become greatly distorted in order that they may perfectly fit the spot of fixation, but I have again and again observed that in old Crepidulas the sole of the foot secretes a calcareous substance by which the animal is so firmly fixed that the foot is often torn to pieces before it can be freed from its attachments. Unlike most prosobranchs, the foot in Crepidula is plainly divided into two portions, a broad and thin *propodium* which is deeply notched in the middle, and a thick, muscular *mesopodium* or sole, by which the animal is attached. The sole of the foot forms a powerful sucker, and when the animal is removed from its attachment so as not to injure the foot, the latter immediately becomes deeply concave on the ventral side, showing that considerable muscular tension was being exerted in order to produce the suction.

C. plana is smaller and much flatter than C. fornicata, and its shell, which is quite fragile, is nearly white in color. It is found most abundantly within those gasteropod shells (Neverita, Lunatia, etc.) inhabited by the larger hermit crab, Eupagurus Bernhardus, and while it may be found in this position either at the outer or inner lip of the shell, it is nearly always so situated that its head is directed toward the opening of the shell in which the crab lives. It is evident that in this case also the Crepidula has taken this position in order that it may be car-

ried about and supplied with food by the hermit, for here again the Crepidula is unable to move about or change its position in the least after it has reached adult size. When a hermit dies, or leaves one shell for another, the Crepidulas in that shell remain attached for some time, but sooner or later perish without attempting to find another shell. Some doubt has been expressed as to whether C. plana is a true species.[1] It has been held that it belongs to the species fornicata, and that those individuals living inside other shells have been slightly modified by their environment, the shell becoming thinner and flatter. There is no doubt, however, that C. plana is a well-marked species, as is shown by its embryological as well as its anatomical differences from C. fornicata.

A very interesting variety of C. plana is found within those gasteropod shells (Illyonassa, Litorina, etc.) inhabited by the smaller hermit crab, Eupagurus longicarpus. This variety resembles the type in all respects save size, being usually less than one-thirteenth the size of adult female specimens found within the larger shells. That this difference in size is not due merely to age is shown by the fact that the dwarfs are sexually mature, and they show by the shape and character of their shells that they are several years old. Apparently, all the organs are perfectly formed, and differ from those of the larger variety only in size. The ova are of the same size as those laid by the larger form, but are fewer in number. The same thing is true of the cells constituting the other organs of the body, so that it may be said that the difference in size between those two varieties is due to the smaller number of cells of which the body of the dwarf variety is composed, rather than to the smaller size of those cells.

There are many evidences that this dwarf form is not a permanent or persistent variety, but only a physiological one.[2] It, like the typical form of this species, is sedentary, and cannot move about after it has reached a certain size. The shape of the shell and body are modified, so that they fit one particu-

[1] Cf. Gould: The Invertebrata of Massachusetts.

[2] It may be doubted whether the word "variety" should be used in this connection at all. However, for lack of a better term, it is employed in its colloquial meaning rather than in a strictly scientific sense.

lar spot and no other ; therefore, the animal cannot migrate to larger quarters after it has grown to its maximum size in the smaller ones. The eggs, embryos, and larvae of the two varieties cannot be distinguished, and since both live together on the same beach, under about the same conditions of food, temperature, and water, it seems probable that the later development of both would be the same if one was not forced by the smaller size of the shell which it inhabits, or by the smaller quantity of food supplied to it, to remain smaller than the typical form. But what is absolutely conclusive is the fact that the dwarfs, when placed in positions where they can obtain a new foothold and increase in size, become almost, if not quite, as large as the common form. A few specimens were found which showed by the shape and character of their shells that for several years they had lived in the shells inhabited by the smaller hermit crab, and had been typical dwarfs; afterward, having been detached, they by rare good fortune gained a new foothold on a larger surface, and their shells began to increase in size, the new portions of the shell becoming shaped so as to fit the surface upon which they had found a new home. In every such shell one can recognize both the dwarf and the normal forms. The dwarfs are what they are by reason of external conditions, and not because of inheritance. In such a case the *shape* and *size* of the body, and the *number of cells* in the entire organism are greatly modified by the direct action of environment. There is no evidence, however, that these modifications of the shape and size of the body and the number of cells have become in the least degree heritable.

C. convexa is smaller than either of the preceding species, and as its name indicates, its shell is more convex, while its color is much darker than either of the others. It is found upon the *outside* of those gasteropod shells (chiefly Litorina and Illyonassa) inhabited by the smaller hermit crab, Eupagurus longicarpus; and it undoubtedly obtains its food, as do the others, from the fragments left by its messmate. Unlike the others, however, it can move about to a limited extent, and, if removed from the surface to which it is fastened, can attach

itself again, though so far as I could observe it never voluntarily leaves the shell upon which it is carried about. It is also said[1] to be found in numbers on blades of eel grass, though I have not seen it in such positions.

C. adunca, a species abundant along the Pacific coast, is remarkably like C. convexa in size, shape, and color of shell, as well as in habits and development. Keep, in his *West Coast Shells*, says of it: "The most common species is C. adunca Sby., hooked slipper shell. The apex is strongly recurved, giving the shell a hooked appearance. Its color is brown, but the deck is white. Living specimens may often be found growing upon rocks or upon other shells. Common length from one-half to three-fourths of an inch. Abundant." Mr. Harold Heath, who has been kind enough to send me specimens of this species, together with material for a study of its embryology, writes me that individuals are found in about equal numbers upon the shells of the "black turban" (Chlorostoma funebrale), and upon shells inhabited by hermit crabs. "The individuals found upon the 'black turbans' seem to come to sexual maturity earlier than upon the hermit shells. Several times on pulling off shells of adunca from the 'black turbans,' I was surprised to find eggs under very small shells, very much smaller than are found with eggs on the hermit crab shells." It seems to me that we have here a case parallel with C. plana and its dwarf variety, though the difference between the two forms in C. adunca is very much less striking than in the case of C. plana. That the phenomena in the two species are similar is still further borne out by the fact that the average number of eggs laid by each individual of C. adunca found upon the "black turbans" is 173.3, while the average number laid by those on hermit crab shells is 201.1. Concerning the habits of C. adunca, Mr. Heath writes: "Their shape indicates that they never leave the spot to which they first become attached. Sometimes surrounded by Bryozoa, the shell is clear within the Crepidula shell. Still, when taken off, they can, and sometimes do, regain a foothold. Many that I placed loose in the aquarium have attached themselves to the 'black

[1] Cf. Gould: The Invertebrata of Massachusetts.

turbans' living there. They appear to breed throughout the whole year."

2. *Breeding Habits.*

The breeding season of C. fornicata lasts on the New England coast from early summer until about August 15. A large proportion of the individuals of this species, examined late in June, were found to have laid their eggs, while none were found with eggs later than the middle of August, though many from widely separated localities were examined. At that time, however, the shells from all these localities were covered by the very small young, or spat, of this species. It may be worth while to remark that the breeding season is always earlier with those individuals found on the shells of Limuli than with those which exist in chains on the sea bottom. This is due, I think, to the fact that in early summer the Limuli are found on shallow, sandy beaches, where the temperature of the water is higher than at a depth of one to six fathoms, where the others are found. The breeding season of C. plana begins somewhat later and lasts longer than that of C. fornicata; several of the former species were found with newly laid eggs as late as September 7. The egg-laying season of C. convexa lasts through nearly the same period as that of C. plana.

As is well known, the sexes are separate in these gasteropods, and the males are fewer in number and smaller than the adult females. Chains of Crepidulas are sometimes found in which there is not a male individual, while isolated females, with from ten to twenty thousand perfectly fertilized eggs, are of frequent occurrence. Considering the sedentary character of these mollusks, the manner of sexual union is an interesting question. There is no doubt that the spermatozoa mingle with the ova before the egg capsules are formed within the oviduct of the female, and yet the mature females are absolutely fixed to one spot, and the largest males have very little, if any, power of movement. The smaller the individual is, however, the greater its power of locomotion. The young of both sexes are freely motile, but as they grow larger they lose this power. In C. plana all the males are much smaller than

the females, and all are motile. In C. fornicata the males may become almost as large as the females, in which case they become immovably fixed to one spot, and cannot, therefore, perform the sexual function unless they are attached near to or upon a female. In C. convexa and in C. adunca all the males are smaller than the females, and are motile. I have carefully taken the volume of a number of alcoholic specimens, and find that the following ratios exist between the males and females of the different species: in C. plana the males are about one-sixteenth the size of the females; in C. adunca, one-eighth; in C. convexa, one-fifth; in C. fornicata, three-quarters. The small males are able to move about more or less freely; if they are detached they readily find a new foothold, and their shells are rarely distorted to fit irregular surfaces, as is the case with the females. There is, then, a marked sexual dimorphism in these mollusks, the mature females being generally much larger than the males; the females are sedentary, the males locomotive, and at the breeding season, or perhaps once for all, the females are visited and fertilized by these motile males. In all mature females, the seminal receptacle, which is a convoluted tubule communicating with the oviduct, is at all times filled with mature spermatozoa. These spermatozoa are attached to the walls of the receptacle by their apices, while their tails project into the lumen exactly as they do in the seminiferous tubules of the male. I believe that the spermatozoa receive nutriment from the walls of the seminal receptacle, and that they can live in this position indefinitely. Since there are myriads of spermatozoa in the receptacle, and furthermore, since none are wasted, so far as I have been able to observe, it might well be that copulation occurs only once in a lifetime.

In C. plana the shell of the male has a characteristic shape, being more nearly round than that of the female, and having a rather sharply pointed apex. This shape is so characteristic that it is generally easy to distinguish a male from an immature female. I have observed a good many cases in which the older part of the shell had the male characters while the newer part was like that of the female. In such animals the penis is

usually very small, and in some cases has almost entirely disappeared. Quite a complete series of stages in the degeneration of this organ was observed, from the fully formed organ on the one hand to a minute papilla on the other. Sections of such animals show that neither male nor female sexual cells are produced at this time. Although the evidence seems to favor the view that we have in these cases an example of successive hermaphroditism, I am not able to assert that this is really the case, although I have spent considerable time in attempting to decide it.

3. *Types of Development in C. fornicata, C. plana, C. convexa, and C. adunca.*

All the ova produced by one individual are laid at about the same time, and the development proceeds very slowly. In C. plana and C. fornicata it is about four weeks from the time the ova are laid until the fully formed veligers escape from the egg capsules, and in C. convexa and C. adunca the period preceding the escape of the young is probably much longer. How long the veligers of the two former species lead a free-swimming life I do not know, since I found it impossible to keep them alive until they were transformed into the spat, or young Crepidulas. From circumstantial evidence, however, I am convinced that in C. fornicata the veligers do not swim about for more than three weeks, probably about two. On July 23, 1890, Mr. Vinal Edwards, collector for the United States Fish Commission, brought me a large number of C. fornicata, dredged from the mouth of the New Bedford river. A large proportion of these were carrying egg capsules, many of which contained fully formed veligers, while most of them were in an advanced stage of development. On August 11, nineteen days later, another lot of Crepidulas were taken at the same place, but no eggs or egg capsules could be found; the parent shells, however, were covered with the very small spat of this species. During July of the next year (1891) I kept a lot of veligers of this species in a wooden box, the bottom of which was covered by silk netting. The box was

anchored in the "codfish pool," a place where there was a large supply of fresh and pure sea-water, and yet where the surface was generally calm. In this box some of the veligers lived for almost two weeks, but although there were stones and shells in the box, I could not find any spat upon them at the end of that time. From these facts it seems probable that the free-swimming life of the veligers lasts not less than two weeks nor more than three. The whole course of development, therefore, from the time the eggs are laid to the close of the larval life and the assumption of adult characters and habits, is from six to eight weeks.

The fertilized eggs in all four species are laid in capsules, which are formed by secretions from the wall of the uterus or nidimental organ.[1] These capsules are united into a bunch, like a cluster of grapes, by a common stem, which is fastened to the shell, stone, or other object upon which the Crepidula lives. This bunch is attached between the two folds of the propodium, and within the mantle cavity of the mother, and since the adults do not move about, it follows that the eggs are always covered by the parent's shell. As a result of this protection the walls of the egg capsules are thin and delicate; very unlike the tough, leathery capsules of most marine prosobranchs. Within the capsules is an albuminous fluid, in which the eggs are immersed, and which is absorbed by the embryos in the course of development. Salensky ('72) has described similar capsules and egg-laying habits in Calyptraea, a sedentary prosobranch nearly related to Crepidula.

The approximate number of capsules and eggs deposited by the mature females of the different species is shown in the following table:

[1] The capsules in Urosalpinx cinerea are marked on the outside by faint spiral lines, and show a tendency to tear in a spiral direction. The same is true of the capsules of Crepidula, though in a less marked degree than in Urosalpinx. This spiral structure is caused, I think, by the rotation of the capsule as it passes through the uterus, in the same way that the spiral character of the egg membranes of birds and reptiles is produced.

TABLE I.

	NUMBER OF CAPSULES.	EGGS IN EACH CAPSULE.	TOTAL NUMBER OF EGGS LAID.
C. fornicata,	55	240[1]	13,200
C. plana (type),	51	176	9,000
C. plana (dwarf),	48	64	3,070
C. convexa,	20	11	220
C. adunca,	10	18	180

These figures are but rough averages made from counting the capsules and the eggs in many of the capsules laid by a large number of mature females; I have no doubt that in another lot of individuals the numbers would be found to vary a little from those given above. In general, however, these figures may be taken as approximately accurate. In all cases the smaller the individual of a species the smaller the number of eggs laid, so that two specimens scarcely ever lay the same number of eggs.[2]

This great difference in the number of eggs laid is the result of the different modes of development in the different species. In no species which is not rapidly increasing or decreasing in numbers are more or less ova produced and fertilized than are just sufficient to insure the continuance of that species in its present numbers. There is no reason to believe that any of these species of Crepidula are rapidly increasing or decreasing in numbers at present; so far as one can judge, each is just about holding its own. If, therefore, one species produces sixty or seventy times as many eggs as another, it must be that in the one case each fertilized ovum has sixty or seventy times as many chances of reaching maturity as in the other case. The history of the development

[1] By a typographical error in a former paper ('92) it is recorded that "about 50 eggs are laid in each pouch or capsule" of C. fornicata. It should read "about 250."

[2] Herrick ('91) showed that the number of eggs laid by the American lobster varies greatly, depending upon the size of the lobster. More recently ('95) he has published an extensive series of measurements of female lobsters and computations of the number of eggs laid by them, from which he constructs the curve of the fecundity of the lobster. He concludes that "the number of eggs produced by female lobsters at each reproductive period varies in a geometrical series, while the lengths of the lobsters producing these eggs vary in an arithmetical series."

in each of these species shows that this is probably the case, for associated with these differences in the number of ova produced are profound differences in the later stages of development. C. plana and C. fornicata pass through a long larval or veliger period, but C. convexa and C. adunca have no free-swimming larval stage at all, the young crawling directly out of the egg capsules in a condition practically adult. Since vast numbers of the free-swimming veligers of C. plana and C. fornicata must be destroyed before reaching that stage of development at which the young of C. convexa and C. adunca first issue from the egg capsules, it is evident that vastly more eggs must be produced by the two former species than by the latter if these different species are to continue in the same relative numbers in which they are now found.

Correlated with the different number of ova produced by the three species are noteworthy differences in the size of the ova, as is shown by the following tables and diagram :

TABLE II.

ABSOLUTE MEASUREMENTS OF THE UNSEGMENTED EGGS OF CREPIDULA. (APPROXIMATE.)

(All measurements were made on eggs preserved in alcohol and mounted in Canada balsam.)

SPECIES.	DIAMETER.	VOLUME.	NUMBER OF EGGS LAID.
C. plana (type),	.136 mm.	.00131709 cu. mm.	9000
C. plana (dwarf),	.136 "	.00131709 " "	3070
C. fornicata,	.182 "	.00315655 " "	13200
C. convexa,	.280 "	.01149406 " "	220
C. adunca,	.410 "	.03608703 " "	180

TABLE III.

RELATIVE MEASUREMENTS OF THE UNSEGMENTED EGGS OF CREPIDULA. (APPROXIMATE.)

SPECIES.	DIAMETER.	VOLUME.	NUMBER OF EGGS LAID.
C. plana (type),	1	1	50
C. plana (dwarf),	1	1	17
C. fornicata,	1½	2⅜	73
C. convexa,	2	8¼	1½
C. adunca,	3	27⅜	1

This dissimilarity in the size of the eggs is due to the differences in the larval history — the species with the most pronounced larval period having the smallest eggs, because but a small amount of nutritive yolk is necessary to carry the development to the free-swimming stage where the larva can take care of itself, while in the species without any larval history enough yolk must be stored in the egg to carry the development clear through to the adult condition.

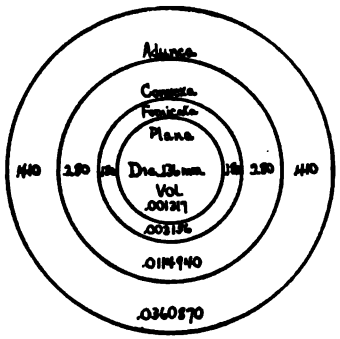


DIAGRAM 1. — Showing the relative size of the eggs of C. plana, C. fornicata, C. convexa, and C. adunca. The actual diameter and volume of each is given in millimeters and cubic millimeters.

The larger size of the eggs in C. adunca and C. convexa as compared with C. fornicata and C. plana, is due chiefly to the greater amount of yolk stored in the entoderm cells of the two former species; and it is worthy of note that this increased quantity of yolk is equally distributed, so that the four macromeres produced by the first two cleavages are nearly equal in size and bear the same relation to each other in the larger eggs that they do in the smaller ones, though in many other molluscan eggs, *e.g.*, Aplysia, Urosalpinx, Unio, and Ostrea, one of the macromeres is very much larger than the other three.

In spite of this vast difference in the size of the eggs in these different species of Crepidula the cleavage, gastrulation, and formation of organs is very similar in all of them. In the large eggs of C. adunca and C. convexa the entoderm cells are, relative to the ectoderm cells, much larger than in C. fornicata, and C. plana; therefore, at the time of the closure of the blastopore there are more ectoderm cells in the large eggs than in the small ones. A count of the nuclei of the ectoderm cells in the species plana, fornicata, and convexa at this stage, shows that they are to each other as two, three, and five respectively, while a comparison of adunca with the other species at an earlier stage (just before the division of the three smaller entoderm cells, see p. 156), shows that it has a larger number of ectoderm cells than either of the others.

The cleavages are precisely the same in all the species up to the 52-cell stage. At this point the ectoderm cells begin to grow more numerous in adunca, though the divisions continue the same until a still later period in the other species.

In all the species the number of mesoderm and entoderm cells remains the same as far as they can be recognized.

There can be no doubt that C. plana and C. fornicata, with their larval types of development, represent a more ancestral condition than C. convexa and C. adunca with their suppressed larval or foetal type.[1] It must be considered that the larval type of development is the more ancestral, from which the foetal type has been derived. The small number of eggs and the direct development of C. convexa and C. adunca are correlated with the small size of the adult in these species, and this in turn may be due to the action of environment through natural selection. These species live upon small objects, chiefly those small gasteropod shells like Litorina or Chlorostoma, which are inhabited by the small hermit crab, and only those individuals could survive in these positions which are small enough to become firmly attached to these shells, while all larger ones would be torn off, and would sooner or later perish. The dwarf variety of C. plana furnishes evidence that the cause here assigned for the small size of C. convexa and C. adunca is not purely imaginary. The ability which all the members of this genus show to adapt themselves to large or small places, and to modify the shell so that it will fit plane, convex, concave or angular surfaces indicates, that the body is very plastic.

But whatever the cause of the smaller size of C. convexa and C. adunca may be, it is evident that the total mass of germinal matter must be less in these than in the larger species, provided that all the other organs are developed in about the same relative proportions, as appears to be the case.

[1] The use of the expression "foetal type of development" in this case is, I think, justifiable. It is true that in these four species various stages in the suppression of the larval development are shown, and even in that species in which the larval development is most completely suppressed, *viz.*, C. adunca, there are many rudiments of larval organs; yet these are only rudiments and they completely disappear before the young escape from the capsules.

Clearly two methods of reducing the total amount of germinal matter are possible : (*a*) the germ cells, while remaining the same in number, may decrease in size, or (*b*) the germ cells may decrease in number, provided a larger proportion of them produce adults. Both of these methods are illustrated within the genus Crepidula. (*a*) The typical form of C. plana, which is about one-third the size of the average C. fornicata, produces almost as many eggs as the latter, but each egg is only about one-third the size of the eggs of C. fornicata. In this case the total amount of germinal matter has been decreased (or increased, according as one or the other species is taken as a standard) by the decrease in *size* of the individual cells. (*b*) In the dwarf variety of C. plana, which is only one-thirteenth the size of the type form, the eggs are of the same size as in the common variety, but much *less numerous*. The method of development in the two varieties is exactly the same, and therefore it follows that unless the typical variety is rapidly increasing in numbers, which does not appear to be true, the dwarf variety must be rapidly disappearing. I think it altogether probable that the eggs laid by the dwarfs are not numerous enough to continue the dwarf variety in its present numbers, and it would rapidly disappear if it were a true or morphological variety. However, since it is merely a physiological variety of C. plana, due to the smaller size of the shell in which the young take up their residence, the continuance of the dwarf variety is not dependent upon the number of eggs produced by the dwarfs ; rather it depends upon the number of the young of C. plana, whether of the common or dwarfed form, which make their abode in the smaller shells.

In C. convexa and C. adunca the amount of germinal matter is reduced in the same way that it is in the dwarfed form of C. plana, *i.e.*, by reducing the number of cells. Since, however, these are true species which are neither rapidly increasing nor decreasing in numbers, it follows that if they produce a smaller number of eggs than the other species, the chances that each egg will produce an adult must be proportionately increased. In C. convexa and C. adunca this is done simply by lengthening the period during which the young organism

remains under the protection of the mother, *i.e.*, by the suppression of the larval type of development.

Since the yolk is almost the only nutriment furnished the young organisms by the mother, it follows that the sooner they can begin to take care of themselves the less yolk will be needed, while the longer they remain in the egg capsules the more yolk will be required. While it is true, therefore, that in the foetal type of development the number of germ cells may be decreased, it is also true that the size of each ovum must be increased. However, in C. convexa and C. adunca the decrease in the number of eggs more than overbalances their increase in volume, so that the total volume of eggs laid is greatly reduced as compared with the other species. The following table gives a basis for comparing the approximate volume of the body of a mature female in each species with the total volume of the eggs laid:

TABLE IV.

COMPARISON OF VOLUME OF ADULT WITH VOLUME OF EGGS LAID.

SPECIES.	RELATIVE NO. OF EGGS.	RELATIVE VOL. OF SINGLE EGG.	RELATIVE TOTAL VOL. OF EGGS LAID.	RELATIVE VOL. OF ADULT FEMALE.
C. convexa,	1½	8½	1	1½
C. plana (dwarf),	17	1	1½	1
C. adunca,	1	27⅔	2½	4½
C. plana (type),	50	1	4½	13½
C. fornicata,	73	2⅔	15	30

The first series of measurements which I made showed a close correspondence between the relative total volume of eggs laid and the relative volume of the adult in these different species. Later and more careful measurements have given the results set down in the above table. The fact is that the sexually mature females of a species vary so much in size, and the eggs laid by them vary so greatly in number, that unless one measures a very great number of individuals of all sizes, no satisfactory ratio between the eggs laid and the volume of the adult can be determined for a given species. However, all measurements and enumerations show that the volume of eggs

laid is, in general, directly proportional to the volume of the adult. This is very plainly the case within a single species where the *number* of eggs laid always stands in direct relation to the size of the animal which lays them. When one species is compared with another this same thing is generally true of the total *volume* of eggs laid, *i.e.*, the species with the largest individuals lays the largest volume of eggs, though the number and size of the eggs in the various species differs immensely. For example, C. convexa, which is about one-twentieth the size of C. fornicata, produces only about one-sixtieth as many eggs of one-fifteenth the total volume of those laid by the latter species. The great reduction in the number and total volume of eggs in C. convexa and C. adunca, as compared with the other species, is made possible by their foetal type of development. At the same time the wide distribution of individuals brought about by the free-swimming veligers of C. fornicata and C. plana is partially secured in C. convexa (I do not know whether this is true of C. adunca or not) by the freely moving young or spat of this species, which are much more active than the spat of either of the other species.

It is very evident that the foetal type of development in C. convexa and C. adunca is correlated with the smaller size of the adult in these species, and for the reasons given above, it seems to me probable that the former may be in some way the *result* of the latter.[1]

4. *General Sketch of the Embryology.*

The First and Second Cleavages. — The chief axis of the ovum corresponds to the future dorso-ventral axis of the embryo.

[1] Although I do not suppose that these relations between the size of the adult and the number, size, and volume of the eggs produced, is a general law applicable to all larval and foetal types of development, neither do I think that such relations are wholly isolated, *i.e.*, true only of this one genus, Crepidula. I believe they will be found to be quite generally true of the gasteropods. Long ago, Fol ('76) called attention to the fact that among the heteropods the smallest species lay the largest eggs. He says, "The smallest heteropods lay relatively the largest eggs, but infinitely fewer than the larger species." He did not observe that the largest eggs had a foetal or suppressed larval development, but I think it would be safe to assume that this is true, and that here also the foetal type, and consequently the larger eggs, are due in part to the smaller size of the adult.

The first cleavage is transverse to the long axis of the embryo, exactly as it is in the case of Teredo, Nereis, and Umbrella, and divides the ovum into an anterior and a posterior half; the second cleavage coincides with the antero-posterior axis of the future embryo, and divides the ovum into right and left moieties. The four macromeres formed by the first two cleavages are nearly equal in size, and each contains the elements of both ectoblast and entoblast, and the left posterior macromere contains, in addition, most of the future mesoblast.

Formation of the Ectoblast. — The whole of the ectoblast is separated from the macromeres by three successive divisions, which separate twelve micromeres from the four macromeres. The four cells first separated from the macromeres constitute the first quartette of micromeres, while those separated by the two following divisions are respectively the second and third quartettes. The first quartette forms the upper hemisphere (umbrella or head vesicle) of the larva, the brain, an apical sense organ, an apical plate of ciliated cells, and a portion of the velum. The second quartette gives rise to the larger part of the velum, the shell gland, and at least a part of the foot. The third quartette I have not been able to follow satisfactorily; its derivatives lie wholly outside of the velar area, and form a considerable part of the lower hemisphere.

Formation of Mesoblast and Entoblast. — Soon after the ectoblast has been segregated, and at the stage when there are twenty-four cells, the left posterior macromere divides obliquely, forming the first member of the fourth quartette, which later comes to lie in the second cleavage furrow at the posterior side of the egg. This cell then divides into right and left portions, and each half again divides into a dorsal and ventral part. The two ventral moieties form a part of the intestine or hinder portion of the alimentary canal. The two dorsal moieties are still mesentoblasts, and the mesoblast is not completely separated from the entoblast until after two more divisions. There are finally formed two mesoblastic teloblasts, each of which gives rise to a mesoblastic band, from which a part of the middle layer is derived. The rest of the middle layer comes apparently from one additional mesoblast cell in

each quadrant, except the left posterior one. These three cells are derived from the advancing edge of ectoblast, and from them the scattered mesoblast cells around the blastopore apparently originate. The other three members of the fourth quartette are purely entoblastic, and they form the lateral and ventral walls of the mesenteron. The residue of the four macromeres is entirely entoblastic, and after they have given rise to a fifth quartette of large yolk cells they form the dorsal wall of the mesenteron.

Gastrulation. — The gastrula is formed by epibole associated with a flattening of the macromeres; there is no invagination. The blastopore closes near the middle of the ventral side, and at this point the mouth soon afterward appears.

The Ectoblastic Cross. — When the stage with forty-two cells has been reached, there appears at the upper pole of the egg a cross of ectoblast cells; the centre of the cross lies exactly at the animal pole, while each of the arms lies between the first and second cleavage planes. Later the whole cap of ectoblast shifts position so that the arms of the cross lie approximately over those cleavage furrows; thus one arm comes to be anterior, one posterior, one right, and one left. In the further development all the arms lengthen, and all save the posterior one divide longitudinally into two parallel rows of cells. All the cells of the cross are derived from the first quartette save the "tip," or terminal cell, of each arm, which comes from the second quartette. A single ectoblast cell, which is at one time the smallest in the egg, but which afterwards becomes the largest, lies in the angle between adjacent arms of the cross. There is one of these in each quadrant, and because of their position and shape they are called for the present the "turret cells." In later stages at least two of them contribute to the formation of the velum.

Change of Axes. — During the later stages of cleavage and throughout gastrulation, the whole of the ectoblast at the upper pole moves gradually forward through an angle of about 90°, so that the centre of the cross, which originally lay at the middle of the future dorsal region, comes to lie at the anterior end of the long axis of the embryo. The entoblast seems to

take no part in this shifting, and the ectoblast on the postero-ventral side of the ovum moves in an opposite direction, *i.e.*, forward on the ventral side. There is thus a stationary point in the ectoblast on the posterior side of the egg, in front of which the ectoblast cells are shoved forward, both on the dorsal and ventral sides. This stationary point is just ventral to the region of the future shell gland, and probably corresponds to the posterior growing-point of the annelids.

Organs formed from First Quartette. — Those cells of the first quartette which lie posterior to the lateral arms of the cross, grow very large and become covered by fine cilia, which protrude through a thin cuticula. These are the cells of the posterior cell plate, and they form the principal part of the walls of a large head vesicle.

The four central or apical cells give rise to an apical sense organ. Each cerebral ganglion is formed at least in part from the cells of the "rosette series" lying on each side of the mid line and between the bases of the anterior and lateral arms of the cross; secondarily the ganglia become connected with the apical organ and with the pedal ganglia and otocysts. The eyes are formed in connection with the cerebral ganglia. All the turret cells lie in the velum, and at least the two anterior ones contribute to the formation of the first velar row. The cells of the lateral arms of the cross divide repeatedly, and some of them form part of the velum. The anterior arm forms a plate of seven large cells reaching from the apical cells to the velum.

Organs formed from the Second and Third Quartettes. — A portion of the velum completely surrounds the first quartette. That part of the first velar row which lies at the ends of the arms of the cross, is formed from the second quartette; the intervening portions, on the anterior side, come from the first quartette (turret cells). The velum is many cell-rows wide, and consists of a preoral and postoral ridge bearing long flagellae and an adoral ciliated groove lying between the two. Dorsally the velum divides into anterior and posterior branches, which are separated by the posterior turrets and the other cells of the posterior plate. The anterior branch runs in on each side toward the apex, and ends on each side of the apical organ;

it traverses the cells of the transverse arms from tip to base. The posterior branch, which is never functional, surrounds the first quartette. When first formed the velar cells are not ciliated, and they lie at the same level as the surrounding cells. Later they are raised into a well-marked ridge, and are finally drawn out into a very extensive wheel-shaped lobe, the long velar cilia being borne around its margin.

The shell gland appears on the postero-dorsal surface just dorsal to the growing-point. It arises as a prominence of ectoderm cells, which from their position seem to be derived from the posterior member of the second quartette. In the place of this prominence an invagination afterward appears ; the margins of the invagination extend rapidly, and a thin cuticle, the first indication of the shell, is secreted by the invaginated cells. As development proceeds the shell becomes asymmetrical, developing more rapidly on the left side than on the right. This asymmetry extends to all the organs posterior to the foot and head vesicle.

The foot arises as a single median protuberance on the ventral side of the body just posterior to the mouth, and in front of the anal or growing region. In later stages the foot becomes more and more prominent posteriorly, until it turns forward and lies ventral to the mouth, though still attached to the body posterior to the mouth.

At the posterior end of the embryo three or four large ciliated anal cells appear very near the growing-point, and at this place the distal end of the intestine is in contact with the ectoderm. The proctodeal invagination does not occur until late in development.

Later Changes. — The intestine is a tube with a distinct lumen, its walls being formed of small cells free from yolk. Its posterior end is formed first, and it grows in length chiefly by the addition of cells at its anterior end, where it opens into the space between the yolk cells. In the course of development, the distal end of the intestine is carried forward on the ventral side, and at the same time the whole hinder portion of the embryo undergoes laeotropic torsion. By the continuance of these two movements the distal end comes to lie in front of the central end, and the latter is found successively on the right

side, the dorsum, and the left side of the embryo. In the end the course of the mesenteron is like a figure 8 open at the top.

In well-advanced embryos the head vesicle and the velar folds become separated by a deep constriction from the posterior part of the embryo. The latter contains all the yolk, and it alone becomes asymmetrical; the head vesicle, velar lobes, and foot, all of which lie anterior to this constriction, retain their bilateral symmetry.

At the point of constriction there is a large spherical prominence on each side, just dorsal to the foot; this is the primitive excretory organ ("urniere").

On the right side of the embryo, just posterior to this constriction, a depression appears in the ectoderm which becomes the branchial cavity.

The formation of the gills, permanent kidney, pericardium, and heart does not occur until a later period than is shown in the figures.

In later stages the head vesicle decreases rapidly in size, the velum is largely, if not entirely, absorbed, the foot becomes relatively very large, and the shell, which during the veliger stage was of the spiral type, takes on the form characteristic of the adult.

In this condition the young or spat resemble the adult forms in all essential respects, and the embryology may be considered as finished.

5. *Abnormalities.*

Under entirely normal conditions all the eggs of C. fornicata and C. plana develop into perfect embryos and veligers (I have not studied C. convexa and C. adunca with reference to this point); still it is not uncommon to find one or two small, abnormal embryos in each egg capsule, even though taken from an individual living in what seems to be a normal environment. But when the adult Crepidulas are removed to the laboratory, and kept in the best possible conditions, the percentage of these abnormalities increases, and when the egg capsules are removed from the mantle cavity of the mother, and kept in dishes of sea-water, the monstrosities increase to such an extent that after a few days not a single normally developing egg or embryo can be found.

These abnormalities may appear in the early stages of cleavage, or they may be found in any of the later stages of development, even up to the fully formed veliger. When present in the early stages the blastomeres are more spherical and less compact than usual. The four macromeres are frequently separated from each other far enough to leave a cavity between them, and into the depression thus formed the overlying ectoderm cells dip down, forming a pit. A similar invagination has been described by Blochmann ('81) for Neritina and by McMurrich ('86) for Fulgur. Both of these authors supposed that this was a normal feature of development, but from the loose character of the cell aggregate and the rounded outlines of each of the cells in Blochmann's figures of Neritina, I believe that the ova there described were segmenting abnormally, and this view is rendered all the more probable when the large proportion (eighty to one) of abnormal as compared with normal eggs in Neritina is taken into account. In fact, Figs. 52–56 of Blochmann's paper represent very well the abnormally segmenting eggs of Crepidula, and I believe the ectodermal pit is in Neritina, as in Crepidula, an abnormal formation.

I have studied the cleavage in Fulgur and find that the ectodermal invagination which McMurrich describes is the shell gland which appears at an early stage, some distance posterior to the apical pole; it is therefore a wholly different feature from the invagination in Neritina, which lies exactly at the apical pole.

Heymons ('93) found that in Umbrella, one of the Opisthobranchiata, the egg capsules contain thirty to forty eggs, and that some of these, though evidently no definite number, do not develop normally. He says: "Von letzeren kommen nicht alle zur normal Entwicklung, indem ein Theil von ihnen gleich nach der ersten Stadien abweichende Verhältnisse und Missbildungen zeigt und später dem Zerfalle unterliegt. Nicht selten sind auch Doppel- oder gar Mehrfachbildungen zu beobachten, die durch Aneinanderwachsen der Eier zu Stande kommen, wie sich im zwei- oder vierzelligen Stadium leicht nachweisen lässt, und die demgemäss auch die doppelte resp. mehrfache Grösse besitzen. Solche Doppelbildungen sind häufig

noch ziemlich spät, noch nach Anlage des Fusses und der Schalendrüse anzutreffen und können bis auf die Berührungsstelle ganz normal ausgebildet sein."

Such double or multiple formations sometimes occur in Crepidula and many other prosobranchs, though so far as I have observed they never reach so advanced a stage as Heymons mentions. Among the later stages in Crepidula the abnormal forms are sometimes nearly like the normal ones, the chief difference being due to irregularities of form, which often take the shape of protrusions or wart-like prominences. A more marked form of degeneration is shown by those embryos which have divided into two or more pieces, each of which may move about independently. It is not an uncommon thing to see a little embryo consisting largely of velum and foot, and entirely disengaged from the yolk cells, swimming actively about in the most amusing fashion. Still greater degrees of degeneration are shown by small fragments, which move about rapidly and are nothing more than little masses of ciliated cells.

It might be considered that in all such cases these abnormal forms were the result of unfavorable conditions, such as imperfect aeration, varying density of the sea-water, or rough mechanical treatment, were it not for the fact that in some forms (*e.g.*, Neritina) even under the most perfectly normal conditions a definite number of abnormalities are always found. McMurrich ('86) has given a pretty complete series of forms showing the varying tendency to produce abnormalities which different species possess. In Fulgur and Urosalpinx all the eggs are said to develop; in Purpurea floridana all do not develop, but a considerable number (not definitely stated) break down and are used as food: in Purpurea lapillus there are five hundred to six hundred eggs in a capsule, only twelve to thirty of which develop, while in Neritina fluviatilis there are seventy to ninety eggs in each capsule, only one of which undergoes regular development. In each of these cases the eggs which do not develop, break down and are used as food by the normal embryos. Such cases cannot be accounted for by assuming merely that the environment is unfavorable. Such a cause would give no such definite results as are said to exist, *e.g.*, in

Neritina. Nor can it in all cases be explained by assuming that in each egg capsule there is a struggle for existence, and that the fittest survive while those less hardy are destroyed, since in some forms, *e.g.*, Neritina, the development does not proceed far enough to introduce such a struggle. From the very beginning of development the ova are divided into two classes, those which segment regularly and develop into normal embryos, and those which divide irregularly and never form embryos at all. Blochmann thinks that in Neritina the eggs which do not develop have not been fertilized, while McMurrich believes that too little yolk was furnished for the number of eggs produced, and that, therefore, some of the eggs broke down and were used as food by the embryos which survived. "This process," he says, "might have been seized upon by natural selection, and increased by it until it became a regular process of development."

I am inclined to believe that in different species different causes may have been operative in producing these abnormal forms. In Neritina, Purpurea and all other forms in which the development of some of the ova goes no farther than a few irregular cleavages, the most probable cause of such non-development seems to be the lack of fertilization, for if McMurrich's supposition is the correct one we should expect to find the ova which undergo development larger than those which do not, but there is no evidence of such disparity in size. On the other hand, in those forms in which the abnormalities do not appear at an early stage and with great regularity, *e.g.*, Crepidula or Urosalpinx, in which they may or may not be present, and if present may occur at any stage, in such cases I am convinced that the abnormal forms are the result of unfavorable environment, *e.g.*, lack of oxygen, presence of bacteria, mechanical pressure, etc.

C. HISTORY OF THE CLEAVAGE.

NOMENCLATURE.

The question of an accurate and convenient nomenclature for the various cells of the cleaving ovum, while of no scientific value, is, nevertheless, of considerable practical importance.

Almost every writer on cleavage has a nomenclature of his own, and not only must one learn a new system every time he reads a new paper, but the difficulties of comparing the work of one author with that of another become constantly greater and greater. If it were possible to invent a system, as some have attempted to do, which would be simple, convenient, and *universally applicable*, it could, and of course would, be accepted by every one who writes upon this subject; but the differences in cleavage are so great that such a consummation seems to me almost hopeless. Besides, there are peculiar features in the cleavage of every egg upon which nature seems to lay emphasis, and such features deserve some special recognition in the nomenclature. Perhaps the most serious objection to any of the systems of nomenclature which have been proposed is the fact that it is almost impossible to recall cells by letters and figures when they differ from each other only in the value of one out of many exponents, *e.g.*, it is practically useless for an ordinary reader to attempt to remember the differences in the position, shape, and history of the cells called b''_2 and b'''_2 of Blochmann's ('81) system, or d^1 and $d^{1.4}$ of Wilson's ('92), whereas it is comparatively easy to recall these cells if they are known as the basal and terminal cells in the posterior arm of the cross. It is not always possible to designate cells by colloquial names which shall be of any help in forming a mental image of them, but wherever it is possible it should be done. At the same time some brief and accurate system of nomenclature is necessary in order to show the derivation of cells, and also for the purposes of comparison and reference.

I have, therefore, concluded to employ, so far as possible, a double system of names for every blastomere, one of which shall be, if you please, its common name, the other its scientific designation. Regarding the latter, which alone needs to be mentioned in this place, I shall, in the main, follow Wilson's system, given in his work on "The Cell Lineage of Nereis," modifying it only to this extent, that the quartettes [1] of cells, separated at various times from the macromeres will be desig-

[1] I use the term *quartette*, as employed by Kofoid ('94), to designate a group of four cells of the same generation, one of which belongs to each of the quadrants

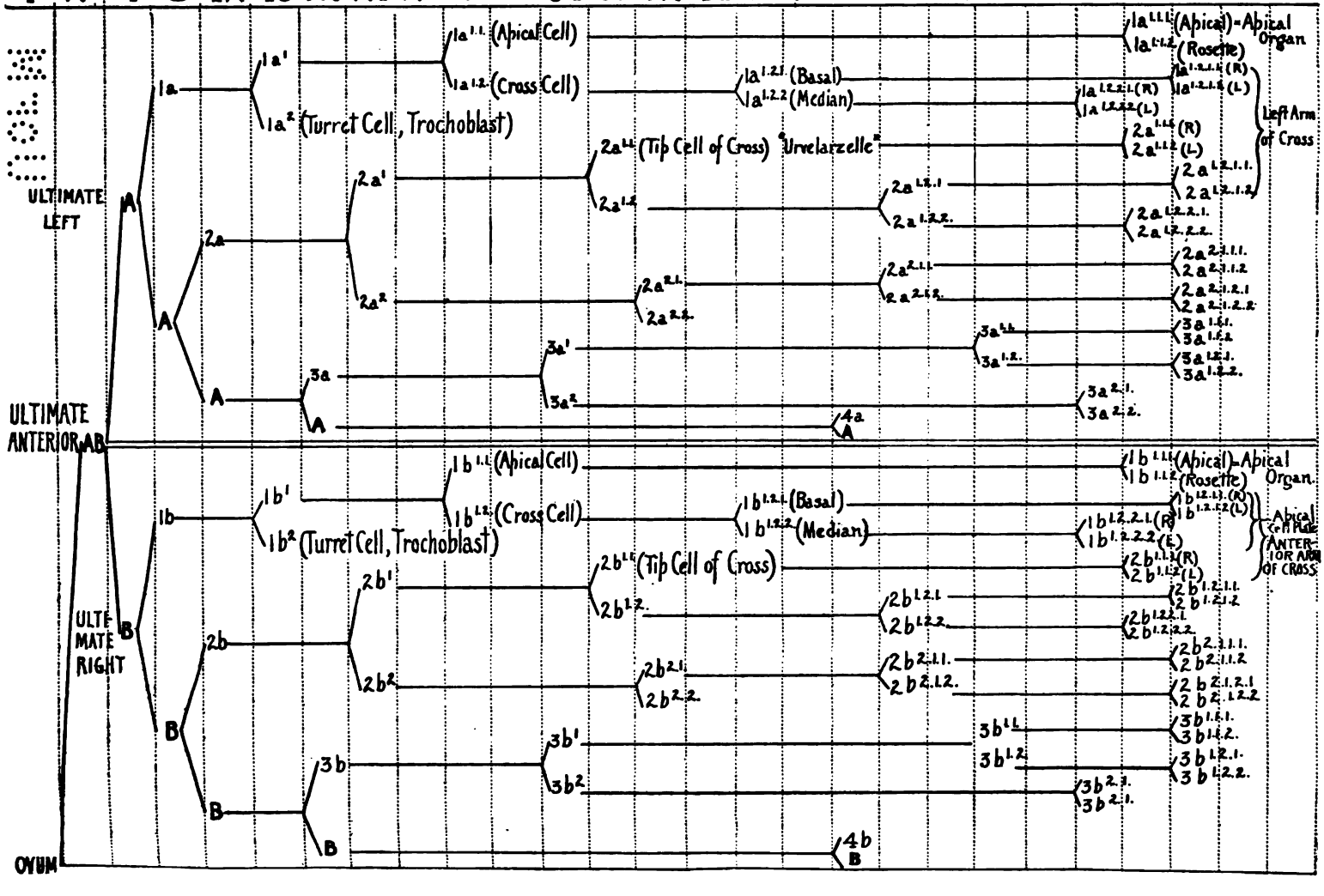
nated by coefficients rather than by exponents ; *e.g.*, the first quartette of micromeres and all their derivatives are designated by the coefficient 1 (1a, 1d, $1a^{1.2}$, $1c^{2.2}$, etc.), the second quartette and its progeny by the coefficient 2 (2a, 2d, $2c^{3.1}$, etc.), the third quartette by the coefficient 3 (3a, 3d, etc.), and the fourth quartette by 4 (4a, 4d, etc.). I emphasize this difference between the quartettes of micromeres because in general their histories are very different, and also because it is only by following the different quartettes that I have been able to trace the cell lineage in the more advanced stages.

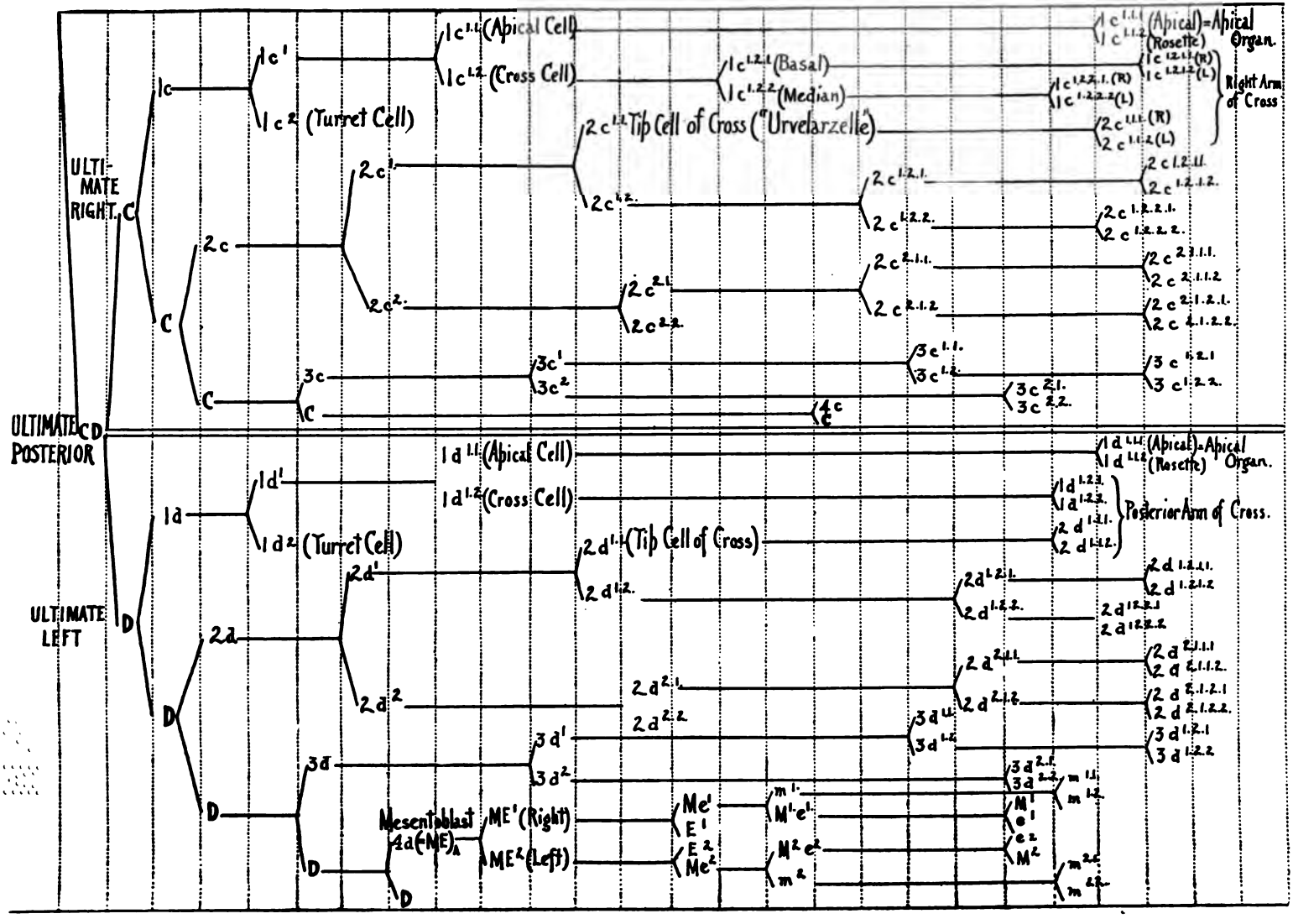
Another and an all-sufficient reason for emphasizing in the nomenclature the different groups or quartettes separated from the macromeres, is the fact that, so far as known, the same number of quartettes with essentially the same destiny is separated in all annelids and mollusks with holoblastic segmentation. This is certainly a feature of great morphological importance, and deserves special recognition in the nomenclature. This system of nomenclature will be better understood by reference to the following cytogenetic table.

The animal and vegetal poles are considered the fixed points in the egg. In the ectoblast the stem or parent cell is in all cases the upper one. The stem cell in the entoblast and mesoblast is in every case the lower one. If, in any case, the cleavage is perfectly meridional (an exceedingly rare thing), the right moiety is considered the stem cell. The terms *right* and *left* are employed in the usual sense, *i.e.*, right is clockwise, left is anti-clockwise. A cleavage is oblique to the right, or, following Lillie ('95), *dexiotropic*, when the upper moiety lies to the right of the lower ; it is oblique to the left, or *laeotropic*, when the upper moiety lies to the left of the lower. The direction of a cleavage refers to the direction of the nuclear spindle, not to the plane of the division wall.

of the egg. In numbering the different quartettes, however, I have departed somewhat from Kofoid's system. The four macromeres are the basal quartette; the first group of ectomeres separated from these are the first quartette, the second group the second quartette, etc.

TABLE OF THE CELL-LINEAGE OF CREPIDULA.




ULTIMATE POSTERIOR
ULTIMATE RIGHT
ULTIMATE LEFT
C
D
1c
1c'
1c²
(Turret Cell)
1c^1.1 (Apical Cell)
1c^1.2 (Cross Cell)
1c^1.2.1 (Basal)
1c^1.2.2 (Median)
2c^1.2 Tip Cell of Cross ("Urvelarzelle")
1c^1.1.1 (Apical)=Apical
1c^1.1.2 (Rosette) Organ.
1c^1.2.1.1 (R)
1c^1.2.1.2 (L)
1c^1.2.2.1 (R)
1c^1.2.2.2 (L)
Right Arm of Cross
2c
2c'
2c².
2c^1.
2c^2.
2c^1.1.
2c^1.1.2
2c^2.1.
2c^2.2.
2c^1.2.1.
2c^1.2.2.
2c^1.2.1.1.
2c^1.2.1.2.
2c^1.2.2.1.
2c^1.2.2.2.
2c^2.1.1.1.
2c^2.1.1.2
2c^2.1.2.1.
2c^2.1.2.2.
3c
3c'
3c².
3c^1.1.
3c^1.2.
3c^2.1.
3c^2.2.
3c^1.2.1
3c^1.2.2.
4c
1d
1d'
1d²
(Turret Cell)
1d^1.1 (Apical Cell)
1d^1.2 (Cross Cell)
2d^1.1 (Tip Cell of Cross)
1d^1.1.1 (Apical)=Apical
1d^1.1.2 (Rosette) Organ.
1d^1.2.1
1d^1.2.2
2d^1.1.
2d^1.2.
Posterior Arm of Cross.
2d
2d'
2d².
2d^1.2.1.
2d^1.2.2.
2d^1.2.1.1.
2d^1.2.1.2
2d^1.2.2.1
2d^1.2.2.2
2d^2.1.1.1
2d^2.1.1.2
2d^2.1.2.1
2d^2.1.2.2.
3d
3d'
3d².
3d^1.1.
3d^1.2.
3d^1.2.1
3d^1.2.2
3d^2.1.
3d^2.2.
m^1.1.
m^1.2.
m^2.1.
m^2.2.
Mesentoblast 4d(=ME)
ME' (Right)
ME² (Left)
Me'
E'
E²
Me²
M'e'
M²e²
m²
M'
e'
e²
M²

THE UNSEGMENTED OVUM (FIG. 1).

The spermatozoa meet the ova in the oviduct and are inclosed with them in the egg capsules, but the maturation and fecundation of the ova do not take place until after the capsules have been laid. I have reserved for another paper the study of the nuclear phenomena which underlie these processes.

Two polar bodies are extruded: they are clear and vesicular, and each contains a small nucleolus-like sphere of chromatin. The chromatin in the first-formed polar body usually divides, though the body itself frequently does not. Both polar bodies remain attached exactly at the centre of the ectodermal area, frequently until the ectoderm cells have extended more than halfway around the egg, Figs. 49 and 50. In all these later stages the chromatin is not surrounded, as in earlier stages, by a clear vesicular layer of cytoplasm, but seems to have dissolved and spread throughout the whole body, so that it stains quite uniformly. Sooner or later the polar bodies fall off and disappear, and in sections of embryos of the stage shown in Fig. 93, I have, in several cases, found them in the mesenteron, having been drawn in with the nutrient fluid surrounding the embryos.

The unsegmented ovum is nearly spherical in C. plana and C. fornicata, though in the larger eggs of C. convexa and C. adunca it is generally elongated in one diameter, so that when seen from either pole the outline is elliptical.[1] The protoplasmic portion in all of these species is small as compared with the yolk, but much smaller relatively in the last two than in either of the first two. There is no sharp boundary line between the protoplasmic and deutoplasmic portions; on the contrary, the former sends out pseudopodia-like branches between the yolk spheres, and the spheres themselves grow smaller and more indistinct as one approaches the protoplasmic portion. Fig. 1, the earliest stage drawn, shows the male and female pronuclei lying close together but still distinct. Each nucleus contains, besides several bands or loops of chromatin, a homogeneously staining nucleolus of considerable size, from which the bands of chromatin seem to radiate. In the figure the female pro-

[1] McMurrich has pointed out that the eggs of Fulgur are elongated.

nucleus is slightly larger than the male, and this continues to be true as long as the pronuclei can be recognized as such.

Two polar bodies are shown in the figure at the upper pole, of which the first formed is much the larger ; the chromatin in this has already divided into two masses, though the cell body is still undivided.

At the vegetal pole of the egg there is frequently found a rounded mass of hyaline substance, which stains homogeneously. It persists until after the first two cleavages, lying in the furrow between the macromeres, but apparently attached to one of them only. I am not satisfied as to the significance of this body, but am inclined to believe that it is a remnant of the stalk of attachment by which the ovum was fastened to the basal membrane of the ovarian follicle. If this view be correct, the polarity of the egg is determined in the ovary, the vegetal pole lying next the membrane, the animal pole next the lumen of the follicle. This is precisely the condition in Unio (Lillie ('95), p. 10), where the point of attachment marks the position of the micropyle. There is no micropyle in Crepidula, and no need of any, since there is no egg membrane, but this hyaline mass suggests the micropyle, not only because it is located at the vegetal pole, and seems to be formed in the same way, but still more remarkably, because the spermatozoan usually, though not invariably, enters the egg at this spot. In all cases the polarity of the egg is definitely established long before the polar bodies are formed, and if my interpretation of the hyaline mass is correct, the animal and vegetal poles of the egg are established at a very early stage in the ovary.[1]

[1] Since the above was written a brief study of the eggs of Fulgur carica and of Sycotypus canaliculatus shows that a similar body, though very much larger than that in Crepidula, is present in these animals. In both Fulgur and Sycotypus this body contains a considerable amount of yolk and yet stains quite uniformly, as it does in Crepidula.

I am convinced that this peculiar body is homologous with the problematical lobe which is described by Mead ('95) in the egg of Chaetopterus, and further, it is probably identical with the polar rings observed by Whitman ('78) in Clepsine, and since then by various authors in different annelids.

I. THE PRIMARY CLEAVAGES.

1. *The First Cleavage. Figs. 2–6.*

I cannot state exactly the length of time which intervenes between fertilization and the first cleavage, nor between the latter and the following cleavages. However, not less than four hours elapse after the entrance of the sperm into the ovum before the first cleavage begins, and the interval is probably longer. I have frequently found Crepidulas in the process of egg-laying, and after carrying the newly laid eggs several miles to the laboratory and there fixing and staining them, have found on examination that the male and female pronuclei were still far apart.

No "segmentation nucleus" is formed, *i.e.*, the male and female pronuclei do not fuse before the appearance of the karyokinetic spindle which introduces the first cleavage. In fact, the male and female chromatin loops remain separate until the equatorial plate stage of the first spindle. About the stage shown in Fig. 2, however, the chromatin loops form a continuous plate; though the part of the plate lying beneath the polar bodies (the upper side in the figure) probably came from the female pronucleus, while the other portion (the lower half) came from the male pronucleus. The axis of the spindle lies in the long diameter of the protoplasmic area, or rather the protoplasmic area continually enlarges its diameter in the direction of the axis of the spindle from the time the spindle first appears until the first cleavage is completed. The radiations of the archoplasmic bodies at the poles of the spindle are plainly visible in all the surface views, and a large central corpuscle or centrosome can be seen in the most favorable preparations. After the chromatin is distributed equally to the two poles of the spindle the division of the cell body begins. A furrow first appears at the formative pole, and gradually extends until it forms a constriction all around the ovum, but deeper at the formative pole than elsewhere, Figs. 3 and 4. The cell body then divides into two equal portions *AB* and *CD*, Figs. 5 and 6. These blastomeres are at first nearly spherical,

and touch each other only by a comparatively small surface; later they become much more closely pressed together, and the surface by which they are in contact becomes much larger, so that each of the blastomeres is almost a hemisphere, Fig. 7.

Immediately after the division of the nucleus the archoplasmic bodies, Figs. 5 and 6, begin to increase in size and to become much more definite in outline. Each one lies close beside the nucleus in the position of the pole of the preceding spindle, and in surface preparations looks as if it might be the shadow of the nucleus. My attention was first called to these bodies by finding what I supposed to be two nuclei in each cell, one of which was fainter in color and outline than the other and looked as if it might be at a lower level in the egg, and it was some time before I could bring myself to believe that these bodies, which are so plainly visible even in preparations of the whole egg, and which in many cases are fully as large as the nuclei themselves, were nothing other than the "archoplasmic bodies" of Boveri or the "spheres attractive" of van Beneden.[1]

A careful study of one of these bodies in its resting stage shows that it is a clear vesicular structure, containing apparently a finely granular fluid and having a fairly definite outline from which radiations proceed in every direction, Figs. 5, 6, 8, 10, etc. As it begins to divide, however, preparatory to the formation of the karyokinetic spindle, the definite outline of the body grows fainter and fainter until it cannot be recognized, while the radiations extend much further through the protoplasm of the cell.

At the close of the first cleavage, the nuclei, asters, and protoplasmic areas lie directly opposite each other in the two blastomeres, Fig. 5, but as soon as the blastomeres begin to flatten against each other and the whole egg assumes a more compact form all these structures move in the direction of a clock's hands, as shown in Fig. 6. *This movement of the nuclei, asters, and protoplasm takes place invariably in the same direc-*

[1] I shall throughout this paper call these bodies the *asters*, a name first used in this connection by Fol ('73) to signify the radiating cytoplasmic structure within the cell.

tion, and it must therefore have been predetermined during, and perhaps before, the first cleavage.

I have not been able thus far to discover by what means or in what manner this movement is predetermined. In Crepidula the first spindle does not seem to indicate any such rotation, though it is exceedingly suggestive to note that Warneck ('50) in the case of Limax and Fol ('75) in Cymbulia found that the first cleavage was oblique to the axis of elongation of the egg. Kofoid ('95) however, in his recent careful work on Limax, found no evidence in favor of Warneck's account. In some cases in which the first cleavage is very unequal, as *e.g.*, in Urosalpinx, the plane of the first cleavage is oblique to the axis of elongation, and it may be that it is also oblique to the polar axis of the egg.

But however the direction of these movements may be predetermined, the fact that they are predetermined, at least during the period of the first cleavage, is a profoundly significant one, indicating as it does that *the first cleavage of the egg belongs to a series of "spiral" cleavages which for at least nine successive generations of cells are alternately dexiotropic and laeotropic.*

Strictly speaking the first cleavage could scarcely be called a *spiral* one, since there is but a single spindle which intersects the chief axis of the egg; and besides there is no definite cross axis to which the direction of this spindle can be referred. It is certain, however, that the dexiotropic turning of the nuclei and protoplasmic areas after the first cleavage is, on the one hand, causally related to their laeotropic turning during the second cleavage, and on the other hand it seems to be predetermined at least as early as the preceding cleavage. It is therefore highly probable that the first cleavage belongs in the same category with the succeeding spiral cleavages, though perhaps it would be more exact and less paradoxical to speak of it as *prospectively spiral and dexiotropic.*

These so-called "spiral" cleavages are always radially symmetrical.[1] A glance at Fig. 6 or 7 will show that the two blastomeres are not mirrored representatives of each other, *i.e.*,

[1] This subject is treated at length in the concluding section of this paper.

the egg is not bilaterally symmetrical with reference to the first cleavage plane, but it is radially symmetrical; the blastomeres are congruent antimeres, and the egg at this stage is a "one-rayed radiate," as Chun ('80) calls the Ctenophores. The radial symmetry of the egg prevails undisturbed from the time polarity is first established (p. 39) until the primary mesoblast is formed (p. 67). After this event the posterior half of the egg becomes more or less bilateral, while the anterior half remains radially symmetrical. Finally, at a relatively late stage the entire egg becomes bilateral.

The rotation of blastomeres in some of the later stages of cleavage has long been known and commented upon. So far as I can find Selenka ('81) first used the term *spiral* in this connection. He described in the polyclades a "laeotropen oder λ-Spirale" in the formation of the first quartette of micromeres, and a "dexiotropen oder δ-Spirale" in the formation of the second quartette, but he did not apply either of these terms to the earlier or later cleavages. Lang ('84) first called attention to the fact that the *second cleavage* in Discocoelis takes place in a "left-wound spiral." Since then this same fact has been observed in the case of many other animals (cf. Conklin ('91), Wilson ('92), Heymons ('93), Lillie ('95), *et al.*), and, with one or two exceptions which will be described later, the direction of this cleavage is invariably the same.

Up to the present, however, no one has shown that the *first cleavage* also is a spiral one. In all other works on this subject, so far as I am aware, it is asserted that the position of the spindles during the second cleavage is the first indication of spiral cleavages (see Wilson ('92), pp. 387, 453, Heymons ('93), p. 249, Lillie ('95), pp. 14, 15).

I believe, however, it may be safely asserted that in all cases in which the second cleavage is laeotropic the first is dexiotropic, and that the initial cause of the spiral cleavages is not to be found in the direction of the nuclear spindles, but rather in the structure of the unsegmented egg itself.

2. *The Second Cleavage. Figs. 7–10.*

The spindles usually appear simultaneously in the two blastomeres, Figs. 7, 9, though occasionally earlier in one than the other, as shown in Fig. 8. The axes of the two spindles are almost parallel to each other, and at right angles to that of the preceding spindle. The two spindles are not quite parallel, however, as is shown in Fig. 7, where the spindles are laeotropic, the left pole in each case being at a higher level in the egg than the right one. Thus the axes of the spindles, when viewed from the side, cross each other at a slight angle. It will also be noticed in Fig. 7 that the entire spindle in each blastomere lies somewhat to the left of the median plane of the blastomere. The position and direction of the spindles in this case indicate, before the division occurs, that the cleavage will be laeotropic. The spiral character of the preceding cleavage could be observed only after the division had occurred.

The first cleavage furrow is at first a straight line as seen from the animal pole, Fig. 6, but as the second cleavage comes on, this line becomes bent slightly to the right when placed in the line of vision, Figs. 7, 9. From the angles where this bent portion joins the rest of the first furrow, the two halves of the second cleavage run outward toward the periphery, Figs. 9, 10. The second cleavage really consists of two quite independent furrows; their ends never meet at the centre, and one of them may appear somewhat earlier than the other, Fig. 8. These furrows begin to form near the animal pole and run out around and through the blastomeres until they reach the vegetal pole, completely dividing the two blastomeres into four, which are approximately equal in size.

3. *The Origin and Significance of the Polar Furrows. Figs. 7–12, Diagram 2.*

The bent portion of the first furrow included between the central ends of the second cleavage is a feature of considerable practical as well as theoretical importance. It is a well-known fact that there is, in the eggs of many animals, a furrow at the intersection of the first and second cleavage planes, which does

not lie in either of these planes, but is oblique to both of them. Rabl ('79) calls this in Planorbis the "cross" or transverse furrow ("Querfurche"), indicating thereby that it lies transverse to the long axis of the embryo. Blochmann ('81) also mentions this furrow as being present in the egg of Neritina, and describes the method of its origin. He calls attention to the fact that it lies in the transverse plane of the embryo; and he considers that it is caused by the difference in the time of division of the two cells. But that this is not generally the case, is shown by the fact that it is present in many eggs in which the division of the first two blastomeres occurs simultaneously. Rauber ('82) has described at some length a similar furrow, which is found in the frog's ovum, as well as in Petromyzon and Gobius. He calls it the breaking line ("Brechungslinie"), and says that it may be formed in two ways: (1) the second furrow really consists of two furrows, one of which divides one of the first two blastomeres, the other the other one; these two furrows may or may not meet in the centre; in the latter case the breaking line is formed; (2) if a breaking line is not formed at first, it may appear later by the shifting of the blastomeres. While Rauber considers that the position of the breaking line has an influence on the subsequent cleavage, he regards its position relative to the other furrows or to the embryonic axes as purely a matter of chance. As he points out, it is particularly well marked in the four-cell stage of many ova; at this stage there are often two "cross furrows" on opposite sides of the egg; these are at right angles to each other, so that each of the four cells is acute at one pole and truncated at the other. O. Hertwig ('80) has also called attention to this furrow in the egg of Sagitta. He says of it: "An dem animalen Pole des Eies, welcher gerade abgebildet ist, stossen nicht alle vier Zellen, wie es bei regelmässiger Furchung der Fall sein sollte, in einem Punkte zusammen, sondern nur zwei derselben berühren sich mit verbreiterten Enden und bedingen eine kurze gerade Furche, welcher wir ihrer Lage nach als *Polarfurche* benennen wollen; die beiden anderen Zellen, welche von der gegenseitigen Berührung ausgeschlossen sind, enden zugespitzt an den beiden

Enden der Polarfurche. Ganz dieselben Verhältnisse wiederholen sich am vegetativen Pole; nur treffen sich hier die beiden Zellen, welche den animalen Pol nicht erreichten, mit verbreiterten Enden. Sie bilden eine *vegetative Polarfurche*, welche die animale, wenn wir beide auf dieselbe Ebene projiciren, unter rechtem Winkel kreuzt, wie man beim Wechseln der Einstellung an dem durchsichtigen Object leicht feststellen kann. . . . Eine ähnliche Anordnung der vier ersten Furchungszellen wie bei Sagitta hat soeben auch Rabl an den Eiern von Planorbis genau beschrieben, er nennt die Polarfurche Querfurche und bemerkt hierzu, dass sie einen wichtigen Anhaltspunkt für die Orientirung des Keimes abgiebt."

In all holoblastic eggs which are laden with yolk the polar furrow at the vegetal pole is much longer than the one at the animal pole, — in fact, the latter may be absent altogether, as is the case with Crepidula. In using the expression "polar furrow" in connection with this animal, it must be understood to refer only to that structure which Hertwig calls "vegetative Polarfurche." As just remarked, the name "Querfurche" seems to have been given in the belief that this furrow is always transverse to the antero-posterior axis of the embryo, as it is in Planorbis and Neritina, and as I have found is the case in Urosalpinx and Tritia. If one may judge from the figures alone this seems to be its position in Nassa and Fusus, as described by Bobretzky ('77), and in Vermetus, studied by Salensky ('87). In all forms, however, in which the first cleavage coincides with the antero-posterior axis, or is at right angles to it, the furrow in question could not be transverse to that axis, but would necessarily be oblique to it; this is its position in Nereis, Umbrella, and Crepidula. In such cases the name "cross furrow" is evidently a misnomer. The furrow bears no constant relation to the axes of the embryo, being at one time transverse and at another oblique to the longitudinal axis; and it is just as illogical to name this furrow from its relation to the axes of the embryo as it would be to name the first cleavage from such a relation, which in some animals coincides with the antero-posterior axis, in others is at right angles to it, and in still others is oblique to it.

There are also objections to the word "Brechungslinie," proposed by Rauber; it is not a breaking line, nor a broken portion of a line, and the name indicates nothing with regard to its position. Moreover the fact that the "Brechungslinie" is not constant in position indicates that it is not the result of a determinate series of spiral cleavages, as is the case among annelids and mollusks, but that it is merely a "pressure surface," the result of surface tension, and it therefore has no reference to the character of the cleavage, which might be radial or bilateral or neither. This term, therefore, even if unobjectionable for the purpose for which it was employed by Rauber, ought not to be applied to the furrow in question.

The expression "polar furrow," however, is open to none of the objections mentioned; this furrow is found only at the two poles of the egg, and so far as the name is descriptive at all, it is quite accurate. I shall, therefore, use it exclusively hereafter to designate that portion of the first furrow which lies between the central ends of the second furrow, both at the animal and vegetal poles. Although always and entirely a part of the first furrow, it seems to lie in, and form a part of, both the first and second furrows.

Although in different animals the polar furrow may bear no constant relation to the embryonal axes, it does in all known cases of spiral cleavage bear a very constant relation to the first and second cleavages. In Crepidula, for example, if the first furrow be placed in the line of vision, the polar furrow always bends to the right, in the second furrow it bends to the left, and this is true whichever end of the furrow is nearer the observer. These relations are true only when the egg is viewed from the animal pole; obviously they would be reversed if seen from the vegetal pole, *i.e.*, the polar furrow would bend to the left when in the first furrow and to the right when in the second. This relation is of great practical importance, since it enables one to distinguish at a glance the first furrow from the second, even up to an advanced stage, and it thus forms a ready means of orientation. In Fig. 10 and all succeeding stages it is impossible to distinguish between the first and second furrows except in this way; in Figs. 8 and 9, how-

ever, the second cleavage is not yet complete, and can, therefore, be easily distinguished from the first, and in the ova which are there figured, as well as in hundreds of others which I have studied, the relation of the polar furrows to the first and second cleavages is always the same.

Similar furrows are shown and described in the works of very many authors, and indeed in the ova of almost every group of animals; but in most cases no mention is made of any definite relation between these furrows and the first and second cleavage planes. In the frog, according to Rauber ('82), this furrow bears no constant relation to the first two cleavages, and Eycleshymer ('95) seems to have found the same thing true of Amblystoma, Petromyzon, and Corregonus. But in a very large number of animals, belonging to groups as far removed from each other as mollusks, annelids, and polyclades, the relation between the polar furrow and the first and second cleavages is a constant one. In Blochmann's figures of the egg of Neritina, and in Lang's figures of Discocoelis, the polar furrow is shown bending to the right in the first cleavage (the position which it has in Crepidula), though neither of these investigators calls attention to this fact in the text or description of figures.[1] The same fact is further shown and commented on by Wilson ('92) in the case of Nereis, Heymons ('93) in Umbrella, and Lillie ('95) in Unio. A very striking exception to this rule has been discovered by Crampton ('94) in the case of Physa, a sinistral gasteropod, in which the direction of the polar furrow is reversed, and he points out the fact that the figures which Rabl ('79) gives for Planorbis, and a figure given by Haddon ('82) for Janthina, seem to show a similar reversal. So far as I know these are the only cases on record in which the polar furrow constantly turns to the left when seen in the first furrow, whereas in *many* cases, as I have indicated, it constantly turns to the right.[2]

[1] One figure which Blochmann gives, Fig. 40, corresponds very closely with my Figs. 9 and 10; the second furrow is still incomplete, and two of the macromeres are much more obtuse at the centre than the other two. The polar furrow thus formed bends to the right in the first furrow just as it does in Crepidula.

[2] Since this was written Kofoid's final paper on Limax ('95) has appeared, in which he thoroughly discusses the "cross furrows," especially the relation of the

A phenomenon so widespread and so striking cannot be wholly adventitious and without significance. As we have seen, Blochmann explains the formation of the polar furrow in Neritina by the fact that one of the first two blastomeres divides before the other one. This would not explain the constant relation of the polar furrow to the first and second cleavages unless in all the groups mentioned one blastomere divided earlier than the other one, and this of course is not the case.

Rauber ('82) attributes the formation of the "Brechungslinie" to a tendency on the part of all the furrows to avoid the pole. This, of course, is not true of the first furrow, and in any case it is no explanation of the phenomenon. Jordan and Eycleshymer ('94) are right when they say (p. 412), "The furrows do not *avoid* the pole ; but the mechanical cell-stresses are rarely so adjusted that the furrows intersect at the pole. There seems no need for a special term — 'Polflucht' — to express this fact, since the 'shunning' of the pole can hardly be a matter of primary significance." But while surface tension is a sufficient causal explanation of such pressure surfaces as the "Brechungslinie," this principle alone is not able to explain the *constant* position of the polar furrow with reference to the first two cleavages, and this constant position is a matter of primary significance.

In his classical work on Nereis, Wilson ('92) has carefully described the polar furrows, and has pointed out the fact that they are of great value in the orientation of the egg and embryo.[1] The position of these furrows is precisely the same in Nereis and Crepidula, except that there is a short polar furrow at the upper pole in Nereis which is generally wanting in Crepidula. In the last section of his paper Wilson points out the significance of the "cross furrow," and although he does not directly explain the cause of its constant relation to

ones on the dorsal and ventral sides of the egg. As my account is in substantial agreement with Kofoid's, and as it touches upon a few points not mentioned by him, I have allowed it to stand as first written.

[1] I had earlier ('91) called attention to the fact that the polar furrow bears a constant relation to the first two cleavages, but had attempted no explanation of this fact.

the first two cleavages, yet that explanation lies so near the surface that I should not take the trouble to enter upon that subject here were it not for the fact that I have a few suggestions to make which are not found in his work.

The polar furrows are in all cases the result of spiral cleavages, and the direction of the polar furrows relative to the first and second cleavages is always dependent upon the direction of the spirals. Because the second cleavage is laeotropic, the vegetative polar furrow bends to the right in the first cleavage and to the left in the second ; in Physa, in which the direction of the spirals is reversed, the direction of the polar furrows is reversed.

The cause of these relations can be made plain by means of the accompanying diagram. In Diagram 2, *a*, the macromeres B and D lie at a slightly lower level than A and C, and have given off A and C by a laeotropic division. It is seen in this figure that there is but one polar furrow, and that it turns to the right when seen in the first furrow, and to the left when seen in the second. This is the state of affairs which prevails in Crepidula, Neritina, Umbrella, Urosalpinx, etc. Let us suppose, however, that the passage from the two to the four-cell stage had taken place in the reverse direction as it does in Physa, and as is shown in Diagram 2, *b*, where A and C lie at a somewhat lower level than B and D, and have given off the latter by a dexiotropic division. There is here but one polar furrow, and when seen in the first furrow it turns to the *left;* when in the second furrow, to the *right.* It is evident, therefore, in all those cases where there is but one polar furrow which turns to the right when seen in the first furrow, and to the left when seen in the second, that the second cleavage was laeotropic.

As a rule when there is but one polar furrow, it is somewhat shorter at the formative than at the vegetative pole, Diagram 2, *c*. Yet as an extreme case there are found ova in which the single polar furrow is almost equal in length at the two poles ; this is admirably illustrated by the egg of C. convexa, Diagram 2, *a*, which is laden with a large quantity of yolk, and in which the macromeres A and C, while lying at a slightly higher level than

B and D, are somewhat smaller in size, while the single polar furrow remains almost as long at the animal as at the vegetal pole. In the egg of C. fornicata, which contains less yolk,

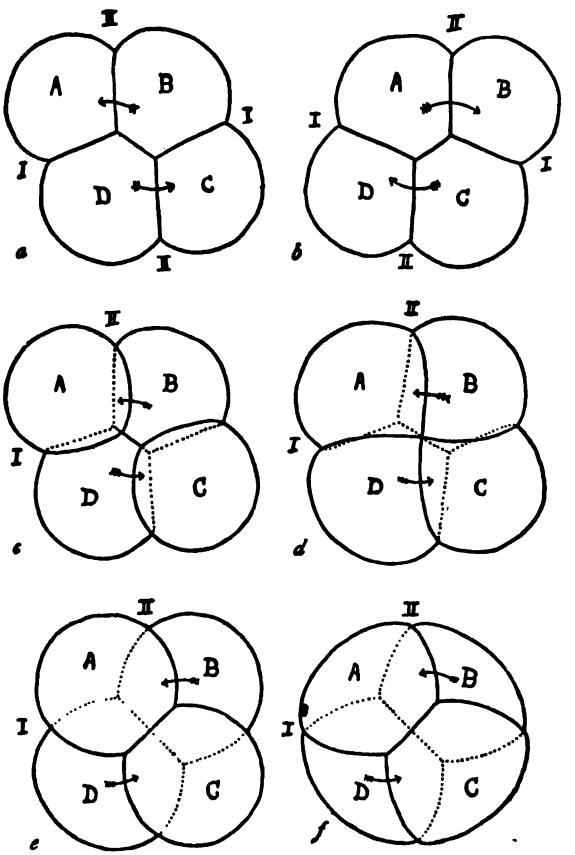


DIAGRAM 2. — Polar furrows of spiral cleavage. — *a*, The condition in eggs with much yolk (C. adunca), in which the polar furrow is as long at one pole as at the other. — *b*, The position of the polar furrow in reversed cleavage. — *c*, The condition in eggs with a smaller amount of yolk (C. fornicata), in which the cells A and C lie at a higher level than B and D, and the polar furrow is shorter at the animal than at the vegetal pole. — *d*, Egg with a still smaller amount of yolk (C. plana), in which the four macromeres meet in a point at the animal pole; there is but one polar furrow, the vegetal. — *e*, Egg of Discocoelis (Lang), in which there are two polar furrows of nearly the same length. — *f*, Egg of Botryllus; polar furrows similar to the last, but whole egg more compact.

and which is represented in Diagram 2, *c*, the relation of the blastomeres to each other is in the main the same as in Diagram 2, *a*, still the macromeres A and C overlie B and D to a greater extent than in the preceding diagram, and there-

fore the polar furrow, while running in the same direction at both poles, is distinctly shorter at the animal than at the vegetal pole. Diagram 2, *d*, represents the condition of the polar furrow in C. plana ; it shows that in this egg, which has less yolk than that of C. fornicata, the blastomeres A and C overlie B and D still more than in the case last mentioned, and that they meet in a *point* at the animal pole. There is here no polar furrow at all at the animal pole, though the one at the vegetal pole is well developed. In Diagram 2, *e*, which is a diagrammatic representation of the egg of Discocoelis as described by Lang, the macromeres A and C not only overlie B and D, but they meet in a line, which forms a polar furrow at the animal pole lying at right angles to the one at the vegetal pole. These furrows may or may not be equal in length ; generally the one at the animal pole is the shorter, though Lillie has found that it is the longer in Unio, which is due to the fact that in this case the cells at the animal pole are larger than those at the vegetal. Finally, in Diagram 2, *f*, which represents the egg of Botryllus, we find the greatest degree of compactness of the blastomeres ; the polar furrows at the upper and lower poles are nearly equal in length, and the individual blastomeres no longer preserve independence of outline, but are rounded into a nearly perfect sphere. Two or more of these different forms may be found at different stages in the cleavage of the same egg. At the moment of cleavage the blastomeres are generally more independent and less compact than during the "resting stages" between cleavages. Thus in many ova the blastomeres at the moment of cleavage are like those represented in Diagram 2, *e*, while during the "resting period" they become much more compact, like those shown in Diagram 2, *f*.

Two types of ova are represented in the diagram given above, one in which there is scarcely any polar differentiation, the other in which it is well pronounced. The former is represented by figures *e* and *f*, and in such cleavage forms polar concentration of protoplasm and nuclei is impossible, the nuclei in fact lie near the centres of the blastomeres and the yolk is uniformly distributed throughout the protoplasm ; the latter type is represented

by figures *a* to *d* in which the polar concentration of protoplasm and nuclei is very marked. In all eggs in which there is but one polar furrow there is decided polar differentiation of the yolk and protoplasm ; where two polar furrows are present this segregation is less pronounced.

The statement that the polar furrow turns to the right when seen in the plane of the first cleavage is true only when there is one polar furrow, and that the one at the lower pole. When there are two polar furrows, as in Diagram 2, *e* and *f*, the lower one still preserves this same relation when seen from the animal pole, while the upper one bends to the *left* when seen in the first furrow, and to the *right* when seen in the second. Of course, if these were viewed from the vegetal pole, the relations would be reversed.

The fact that in very many cases the first cleavage is dexiotropic and the second cleavage laeotropic is a profoundly important and significant one, determining as it does, not only the direction and relation of the polar furrows, but *also influencing more or less the character and direction of every succeeding cleavage. They are the first of a long series of spiral cleavages which take place alternately to the right and to the left, each of which, except the first, finds the sufficient cause of its direction in the direction of the preceding cleavage.*

4. *The Axial Relations of the First Two Cleavages.*

Throughout the course of segmentation the four macromeres remain very much larger than the cells to which they give rise, and as they do not change their relative position, at least until about the time of the closure of the blastopore, it becomes very easy to orient all the future furrows and cells with reference to the first two cleavages. If we examine one of the later stages, such as Figs. 61 and 64, in which the antero-posterior axis of the embryo is well marked by the elongated blastopore, we find that the four macromeres and the polar furrow are still recognizable, and that the cleavage line in which the polar furrow bends to the right, *i.e.*, the first cleavage, is transverse to the antero-posterior axis of the